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**Original Research Article**

# Stability Indicating RP-HPLC Method for Bosentan in Tablet Dosage form

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## Abstract

A simple, precise and reproducible stability indicating HPLC method has been developed and validated for determination Bosentan on an Intersil ODS column (250*4.6*5um) using a mobile phase consisting of Disodium phosphate Buffer and acetonitrile (30:70) at flow 1.0 ml/min. Detection was carried out at 273nm. The method was validated with respect to specificity, accuracy linearity, precession, ruggedness and robustness parameters as per ICH guidelines. Linearity of the method was found to be 0.999; % RSD of precession was below 2.0%, recovery of added drug within 98-102 %, System suitability parameters were within limit for Bosentan. The Degradation study was performed during different stress conditions in stability studies. The results obtained in this research work are indicated that the developed method is simple, accurate and precise, sensitive and applicable for analysis in commercially available formulation sample.

**Keywords:** Validation, ICH, RP-HPLC, Stress degradation study.

## 1. Introduction

Bosentan is chemically known as 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide, monohydrate, It is a selective endothelin-receptor antagonist. There Mechanism of action Bosentan is a competitive antagonist of endothelin-1 at the endothelin-A (ET-A) and endothelin-B (ET-B) receptors. Under normal conditions, endothelin-1 binding of ET-A or ET-B receptors causes constriction of the pulmonary blood vessels. By blocking this interaction Bosentan decreases pulmonary vascular resistance. Bosentan has a slightly higher affinity for ET-A than ET-B [1-3]. Various spectrophotometric methods have been reported for the determination of Bosentan in pharmaceutical tablets using different reagents; UV spectrophotometric and HPLC method were developed [4-10].

The aim of the present work was to develop HPLC method for estimation of Bosentan from its Pharmaceutical Preparation. Therefore it is desirable to develop simple and reproducible analytical methods for used in analytical application, there should be more consistency in validation practice with key analytical parameter, including System Suitability, accuracy, precision, specificity, limit of detection, limit of quantization, linearity, range, ruggedness, robustness, stability [11].

## 2. Material and Methods

Bosentan working standard were received as gift sample from Spectrum Lab Hyderabad. The marked sample Bosentas (62.5mg) was used for analysis purpose. Water (HPLC grade), HPLC grade Acetonitrile Methanol (Merck) and AR grade Disodium Hydrogen Phosphate, Sodium Hydroxide, Hydrogen Peroxide (RANKEM) was used. Mili Q water used in mobile phase and diluent preparation. Chromatographic separation performed on Waters® liquid chromatographic system equipped with PDA detector, with a 20ul injection loop volume by using M power software.

**2.1 Preparation of Buffer**

Weighed and transferred 1.41gms of Di sodium hydrogen phosphate in to a 1000ml of Mili-Q water.

**2.2 Preparation of Mobile Phase**

Mixed Phosphate Buffer: Acetonitrile in the ratio of 30:70%v/v and sonicated to degassed for 10 minutes.

**2.3 Preparation of Diluent**

Mixed water: Acetonitrile in the ratio of 50:50 %v/v and sonicated.

**2.4 Chromatographic conditions**

The Chromatographic separation were achieved by using Intersil ODS C18 column (250*4.6*5um) analytical column. The mobile phase consisting of phosphate buffer: Acetonitrile in (30:70) at flow 1.0 ml/min. Detection was carried out at 273nm and with isocratic program run time was 6 min. The column Oven temperature was 30°C and ambient sampler temperature.

**2.5 Preparation of standard solution**

Weighed and transferred 31.25 mg of standard into a 25ml volumetric flask, methanol was added and sonicated to dissolved and made up to the final volume with methanol. Transferred 1ml of solution in to a 10ml with diluents.

**2.6 Preparation of sample solution:**

Five tablets (Bosentas) were weighed and crushed to fine powder, uniformly mixed. The powder equivalent to one tablet weight was taken in to 100 ml volumetric flask and added diluent, sonicated for 30 min with intermediate shaking and made up to mark with diluent. Pipetted out 2ml of this solution in to 10 ml volumetric flask and made to mark with diluent, mixed well.

## 3. Results and discussion

Method development trial was taken by changing mobile phase, column, flow rate, column temperature, and buffer composition. Colum was selected based on the column chemistry and get good peak shape, and pass system suitability parameter. The mobile were optimised so that there were no interferences of diluent and excipients. The method were finalized with phosphate buffer: Acetonitrile (ratio 30:70%), column Intersil ODS C18 column (250*4.6*5um) and on PDA detector 273nm maxima used for analysis purpose.

**3.1 Validation of developed method**

The method was validated for method precision, linearity, accuracy, precision, and specificity study as per ICH norms. All the validation studies were carried out by replicate injection of the sample and standard solutions.

The diluent (Blank) and standard were injected to HPLC system; it was observed that there were no interferences of blank with standard peak as shown in fig 1 and fig 2. The retention time obtained 2.70 min.

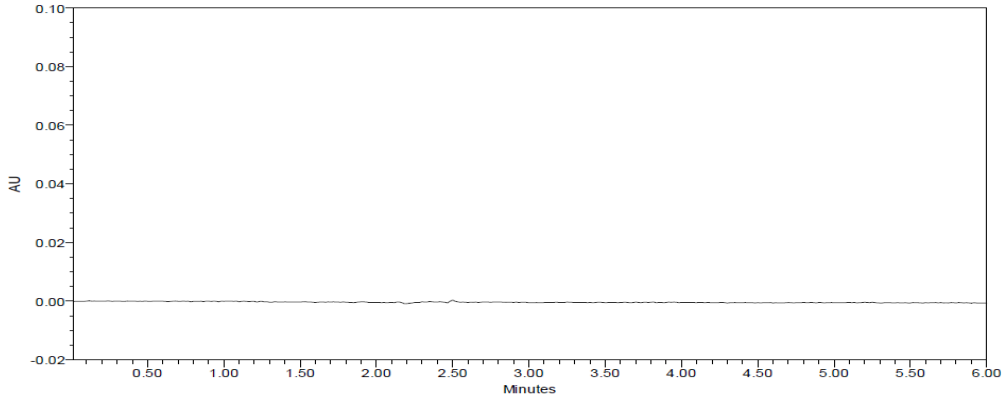


**Fig 1: Blank Peak**

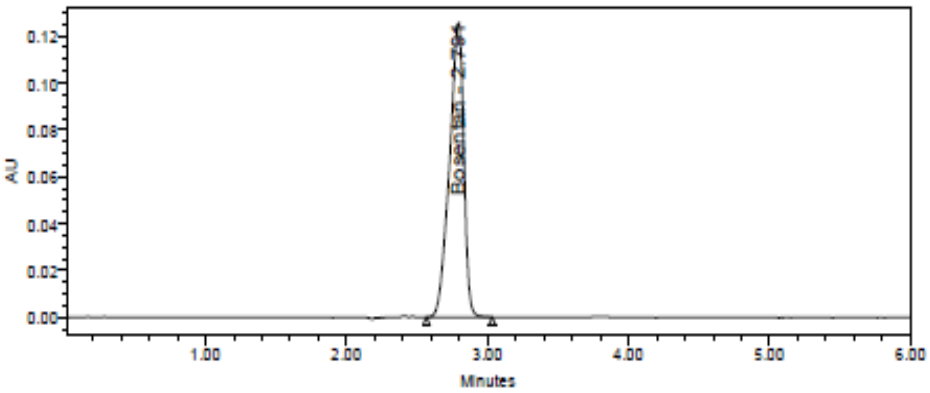


**Fig 2: Bosentan standard peak**

**3.2 System Suitability**

Six replicates injection of standard solution was injected into the chromatograph as per methodology. The results of system suitability of Bosentan is as follows Table 1.

**Table 1: System Suitability results of Bosentan**

| Parameters | Observation |
|---|---|
| Theoretical plate | 3427 |
| Tailing Factor | 1.02 |
| % RSD of the area of replicate injection | 0.14 |

**3.3 Calibration curve:**

Linearity of method was developed from 25 - 175 µgmL-1 for Bosentan. The linear regression equations, y = 91130x+1250 and correlation coefficient was found to be 0.999. Where y is response (peak area) and x is the concentration.

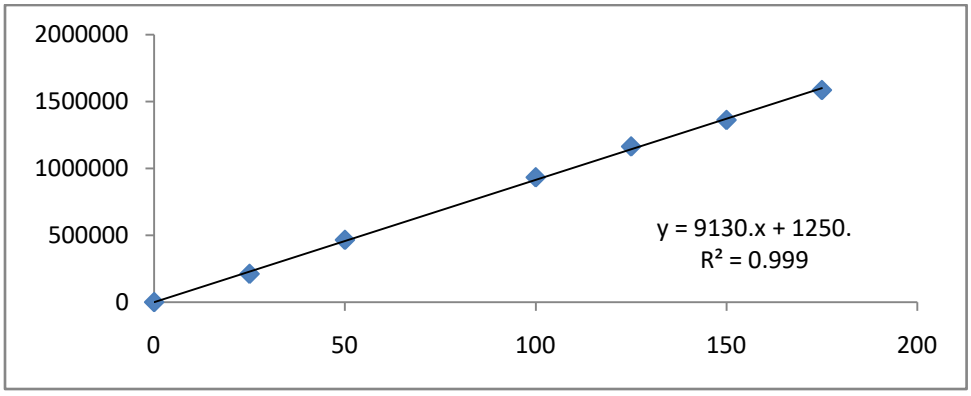


**Figure 1: Calibration curve of Bosentan**

**3.4 Accuracy**

Accuracy of developed method was confirmed by doing recovery study as per ICH norms at three different concentration levels 50%, 100% and 150% by replicate analysis (n=3). The result of accuracy study was reported in Table 2. From the recovery study, the results were within the range of acceptance i.e. %RSD<2.

**Table 2: Accuracy study data of Bosentan**

| % Recovery of Level | | Amount Added (in ppm) | Amount Recovered (in ppm) | % Recovery | Mean Recovery | %RSD |
|---|---|---|---|---|---|---|
| **50%** | 1 | 62.5 | 63.33 | 101.33 | 100.54 | 0.68 |
| | 2 | 62.5 | 62.62 | 100.20 | | |
| | 3 | 62.5 | 62.56 | 100.10 | | |
| **100%** | 1 | 125 | 127.00 | 101.60 | 100.78 | 0.52 |
| | 2 | 125 | 125.82 | 100.66 | | |
| | 3 | 125 | 125.09 | 100.07 | | |
| **150%** | 1 | 187.5 | 190.36 | 101.53 | 100.23 | 0.36 |
| | 2 | 187.5 | 189.87 | 101.22 | | |
| | 3 | 187.5 | 186.49 | 99.46 | | |

**3.5 Precision, Limit of Detection, and Limit of Quantitation**

The concentrations of drugs were measured on the same day with different six preparations and with different day, different column and another six preparations for inter day study. The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of standard and S is the slope of the calibration curve. The results are reported in Table 3.

**Table 3: Precision and Inter Day Precision, LOD and LOQ Studies**

| Drug | Method Precision %RSD (n=6) | Inter day Precision %RSD (n=6) | LOD (ppm) | LOQ (ppm) |
|---|---|---|---|---|
| Bosentan | 1.35 | 1.08 | 0.09 | 0.19 |

**3.6 Robustness**

Robustness of the method was demonstrated by deliberately varying the chromatographic condition such as Flow rate changed by ± 10% (i.e. 0.9mL/min and 1.1mL/min) Column temperature changed by ± $5^0$C ($30^0$C i.e. $25^0$C and $35^0$C). Mobile phase composition changed by absolute ±2% variation (i.e. Buffer: Acetonitrile 670:330 and 630:370). The results were tabulated as in Table 4.

**Table 4: Robustness data for system suitability of Bosentan**

| Robustness Parameter | Theoretical plates | Tailing Factor | %RSD |
|---|---|---|---|
| **Limits** | **NLT 2000** | **NMT 2.0** | **NMT 2.0** |
| Low Flow | 3180 | 1.12 | 0.24 |
| High Flow | 2594 | 1.12 | 0.40 |
| | | | |
| Low Organic | 2252 | 1.05 | 0.30 |
| High Organic | 2686 | 1.01 | 0.35 |
| | | | |
| Low Temperature | 2248 | 1.02 | 0.42 |
| High Temperature | 2553 | 1.21 | 0.73 |

**3.7 Degradation Study**

Specificity covers the degradation of drug product in Acid degradation, Base degradation, Peroxide degradation, Neutral (reflux condition) Thermal degradation and Photo degradation. All degradation conditions with % degradation and peak purity were mention in table 5.

**Table 5: Forced degradation data**

| Degradation condition | %Assay | % Total degradation | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| **AS such** | 100.71 | NA | NA | NA |
| **Acid degradation (2N_HCl/60ºC/30min.)** | 96.72 | 3.99 | 0.128 | 0.332 |
| **Base degradation (2N_NaOH/$60^0$C/30min.)** | 97.91 | 2.8 | 0.123 | 0.333 |
| **Peroxide degradation (20%$H_2O_2$/$60^0$C/30min.)** | 95.14 | 5.57 | 0.130 | 0.355 |
| **Neutral degradation ($H_2O$_60ºC /6 hrs.)** | 99.23 | 1.48 | 0.144 | 0.331 |
| **Thermal degradation (105 ºC /6hrs.)** | 99.19 | 1.52 | 0.128 | 0.338 |
| **Photo degradation (1.2million lux hours)** | 99.36 | 1.35 | 0.116 | 0.330 |

For purity: Purity Angle < Purity Threshold

The developed method was found specific and selective, as there was no interference of blank and individual drug. Also method was found stability indicating based on degradation study, it was found to be sensitive to acidic, basic, peroxide conditions. No significant degradation was obtained under any of above mentioned stress conditions.

## 4. Conclusion

A new, reversed-phase HPLC method has been developed for analysis Bosentan in a tablet formulation. It was shown above that; the method was linear, accurate, reproducible, repeatable, precise, robust, specific and stability indicating proving the reliability of the method. The run time is relatively short, as method is stability indicating which enable rapid determination of many samples in routine and quality control analysis of tablet formulations. Hence, the proposed method was successfully applied to analyze preparation containing Bosentan.

## Acknowledgement

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